

Relative Adjuvant Efficacy of Al(OH)₃ and Saponin Is Related to the Immunogenicity of the Antigen

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Al(OH)₃ is the most widely used adjuvant in human and veterinary vaccines [1]. Saponin, a mixture of triterpene glycosides extracted from the bark of the Quillaia tree [2], is included in foot-and-mouth disease vaccines [2] and is also very effective in experimental vaccines against protozoal parasites [3]. The two adjuvants differ markedly in their relative efficacy depending on the antigen. The humoral response to bovine serum albumin (BSA) is better potentiated by Al(OH)₃ than saponin, whereas the opposite is the case for sheep red blood cells (SRBC) [4].

This differential adjuvant activity may be an expression of underlying differences in the mechanism of action of the adjuvants. It could depend on the intrinsic immunogenicity of the antigen, SRBC being potent immunogens, whereas BSA is readily tolerogenic [5]. This communication presents the results of experiments designed to provide further evidence for this hypothesis, using as antigens BSA and keyhole limpet haemocyanin (KLH), and DNP conjugates of BSA, fowl gamma globulin (FGG) or KLH. BSA, FGG and KLH represent an ascending series of antigenic strength in terms of their ability to elicit T-cell mediated help [6].

The potentiation of the primary humoral response to BSA or KLH by Al(OH)₃, saponin, or a mixture of the two adjuvants was compared (table I). The effect of combining the adjuvants was tested in order to find out if BSA would become susceptible to the adjuvant action of saponin if it were insolubilized by adsorption onto Al(OH)₃. Saponin does not interfere with the uptake of ¹²⁵I-BSA by Al(OH)₃ (data not shown). The selective efficacy of Al(OH)₃ for BSA was confirmed, but its adjuvant effect was inhibited rather than enhanced by the addition of saponin. In contrast, the response to KLH was better potentiated by

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Table I. Al(OH)₃ and saponin as adjuvants for KLH and BSA

Immunization	Day 28 Ab response	
	anti-KLH (RIA endpoint)	anti-BSA (log ₁₀ ABC, mean \pm SD, n = 5)
Antigen	10	-0.53 \pm 0.54
Antigen + Al(OH) ₃	80	1.33 \pm 0.31
Antigen + Saponin	640	-0.35 \pm 0.43
Antigen + Al(OH) ₃ + Saponin	1,280	0.14 \pm 0.44

Mice were immunized s.c. with 2.5 µg KLH (Pacific Biomarine Labs.) or BSA (Armour) alone or plus 100 µg (of Al) Al(OH)₃ (al-hydrogel, Superfos) or 50 µg saponin (Food Industries Ltd.). The responses was measured by a solid phase radioimmunoassay [8] for KLH and the Farr test [9] for BSA.

saponin than Al(OH)₃ and the two adjuvants interacted positively rather than negatively.

The comparison of Al(OH)₃ and saponin was extended to the humoral response to DNP coupled to BSA, FGG or KLH. The relative efficacy of the adjuvants depended on the carrier, Al(OH)₃ being superior to saponin with DNP-BSA and DNP-FGG, and the opposite with DNP-KLH (table II). The effect was not correlated with the concentration of antigen or the titre of anti-DNP antibody. Thus, the response to DNP-KLH remained selectively susceptible to saponin even when the dose of DNP-KLH was reduced to 0.1 µg, at which level the anti-DNP response (plus saponin) was very close to that elicited by 1 µg of DNP-BSA or DNP-FGG plus Al(OH)₃, and considerably less than that with 10 µg of the latter antigens.

These results strengthen the evidence in favour of the hypothesis that the selective adjuvant efficacy of Al(OH)₃ and saponin depends on the immunogenicity

II. Relative efficacy of Al(OH)₃ and saponin as adjuvants in DNP-BSA, DNP-FGG and DNP-KLH

Dose μg	Day 28 antibody response (RIA endpoint)		
	no adjuvant	Al(OH) ₃	Saponin
SA	<10	320	<10
	10	<5,120	1,280
GG	<10	640	<10
	10	<2,560	80
LH	0.1	>10	10
	1	>10	40
	10	160	2,560
			20,240

DNP-BSA (11 DNP/mol), DNP-FGG (12 DNP/mol) or DNP-KLH (1 DNP/10⁶ daltons) were injected s.c. alone or plus 100 μg Al(OH)₃, or 50 μg saponin. The response was measured by radioimmunoassay [8], using DNP-BSA plates for the DNP-FGG and DNP-KLH sera, and DNP-KLH plates for the DNP-KLH sera.

he antigen. In themselves, they do not completely exclude a role for the molecular weight (MW) of the antigen, since KLH (MW > 10⁶) is larger than BSA (50,000) or FGG (MW 160,000). However, re-observations using human growth hormone as antigen enable this factor to be discounted; Al(OH)₃ or saponin potentiated the humoral response in responder CBA mice, whereas saponin is the best adjuvant in high responder Balb/c mice (Bomford, unpubl.). The physical nature of the antigen will still be important for the adjuvant action of saponin for SRBC and other cellular antigens which contain membrane cholesterol, to which saponin can form a highly immunogenic complex [7].
 The recognition that differences in relative adjuvant activity are related to antigenic strength offers a basis for the investigation of the mechanism of adjuvant action, and is also important in the selection of adjuvants for experimental immunization or vaccination.

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